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TITLE: Recoding of DNA sequences permitting expression in yeast and obtained transformed yeast

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CLAIMS:

What is claimed is:
1. A method of producing a protein in yeast comprising:
(a) selecting a protein encoded by a reference DNA sequence that comprises at least one region of ten consecutive codons comprising at least two codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons;
(b) replacing at least 50% of codons that have a first frequency of use codons in the reference DNA sequence with having a second frequency of use by yeast of greater than or equal to 15 per 1000 codons and encoding the same amino acid as the codon being replaced to provide a modified sequence;
(c) transforming yeast with a vector comprising said modified sequence under the control of yeast regulatory elements; and
(d) culturing said transformed yeast under conditions whereby said modified sequence is expressed to provide said protein.
2. The method of claim 1 wherein said first frequency of use is less than or equal to 12 per 1000 codons.
3. The method of claim 1 wherein said first frequency of use is less than or equal to 10 per 1000 codons.
4. The method of claim 1 wherein said codons having said first frequency of use are each independently selected from the group consisting of CTC, CTG and CTT encoding leucine, CGG, CGC, CGA, CGT and AGG encoding arginine, GCG and GCC encoding alanine, GGG, GGC and GGA encoding glycine and CCG and CCC encoding proline.
5. The method of claim 1 wherein said codons having said first

frequency of use are each independently selected from the group consisting of CTC and CTG encoding leucine, CGG, CGC, CGA, CGT and AGG encoding arginine, GCG and GCC encoding alanine, GGG and GGC encoding glycine and CCG and CCC encoding proline.
6. The method of claim 1 wherein said second frequency of use is greater than or equal to 18 per 1000 codons.
7. The method of claim 1 wherein said second frequency of use is greater than or equal to 20 per 1000 codons.
8. The method of claim 1 wherein said region of ten consecutive codons comprises at least three codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons.
9. The method of claim 1 wherein said region of ten consecutive codons comprises at least four codons that have a first frequency of use by yeast of less than or equal 13 per 1000 codons.
10. The method of claim 1 wherein said region of ten consecutive codons comprises at least five codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons.
11. The method of claim 1 wherein said region of ten consecutive codons comprises at least six codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons.
12. The method of claim 4 wherein at least 50% of said codons that have a first frequency of use are replaced, the replacement selected from CTC, CTG or CTT replaced by a codon independently selected from TTG and TTA; CGG, CGC, CGA, CGT or AGG replaced by AGA; GCG or GCC replaced by a codon independently selected from GCT and GCA; GGG, GGC or GGA replaced by GGT; and CCG or CCC replaced by CCA.
13. The method of any one of claims 1 and 8-11 wherein at least two of said codons that have a first frequency of use are adjacent.
14. The method of any one of claims 8-11 wherein at least three of said codons that have a first frequency of use are adjacent.

15. A method of producing a protein in yeast comprising:
(a) selecting a protein encoded by a reference DNA sequence that has at least one region of ten consecutive codons comprising at least two codons encoding leucine and independently selected from CTC and CTG;
(b) replacing at least 50% of said codons encoding leucine by a codon independently selected from the group consisting of TTG and TTA to provide a modified sequence;
(c) transforming yeast with a vector comprising said modified sequence under the control of yeast regulatory elements; and
(d) culturing said transformed yeast under conditions whereby said modified sequence is expressed to provided said protein.
16. The method of claim 15 wherein at least 50% of said CTC and CTG codons are replaced by TTG.
17. The method of claim 15 wherein said region of ten consecutive codons comprises at least three codons encoding leucine and independently selected from CTC and CTG.
18. The method of claim 15 wherein said region of ten consecutive codons comprises at least four codons encoding leucine and independently selected from CTC and CTG.

19. The method of claim 15 wherein said region of ten consecutive codons comprises at least five codons encoding leucine and independently selected from CTC and CTG.
20. The method of claim 15 wherein said region of ten consecutive codons comprises at least six codons encoding leucine and independently selected from CTC and CTG.
21. The method of claim 1 or 15, wherein at least 20% of the total codons of said reference DNA sequence are codons that have a frequency of use by yeast of less than or equal to 13 per 1000 codons.
22. The method of claim 1 or 15, wherein at least 30% of the total codons of said reference DNA sequence are codons that have a frequency of use by yeast of less than or equal to 13 per 1000 codons.
23. The method of claim 1 or 15, wherein said region of ten consecutive codons is in the 5' region of said reference DNA sequence.
24. The method of claim 1 or 15, wherein said reference DNA sequence is of plant origin.
25. The method of claim 1 or 15, wherein said protein is an enzyme.
26. The method of claim 1 or 15, wherein said protein is cytochrome P450.
27. The method of claim 1 or 15, wherein said yeast is selected from the group consisting of Saccharomyces, Kluveromyces, Hansenula, Pichici and Yartowia.
28. The method of claim 1 or 15, wherein said yeast is S. cerevisiae.
29. The method of claim 24 wherein said plant is a monocot.
30. The method of claim 24, wherein said plant is selected from the group consisting of wheat, barley, oats, rice, maize, sorghum and cane sugar.
31. The method of claim 26, wherein said reference DNA sequence comprises SEQ ID NO: 1 or 10.
32. The method of claim 26, wherein said modified DNA sequence comprises SEQ ID NO: 7, 8, 9 or 14.
33. A method of producing a protein in yeast comprising:
(a) selecting a protein encoded by a reference DNA sequence that comprises at least one region of ten consecutive codons comprising at least two codons independently selected from the group consisting of CTC, CTG and CTT encoding leucine, CGG, CGC, CGA, CGT and AGG encoding arginine, GCG and GCC encoding alanine, GGG, GGC and GGA encoding glycine, and CCG and CCC encoding proline;
(b) replacing at least 50% of codons in one region of ten consecutive codons, the replacement being selected from CTC, CTG and CTT replaced by a codon independently selected from TTG and TTA; CGG, CGC, CGA, CGT and AGG replaced by AGA; GCG and GCC replaced by a codon independently selected from GCT and GCA; GGG, GGC and GGA replaced by GGT; and CCG and CCC replaced by CCA.
34. A method of preparing a DNA sequence having improved translation in yeast comprising:
(a) selecting a protein encoded by a reference DNA sequence that comprises at least one region of ten consecutive codons comprising at least two codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons;

(b) replacing at least 50% of codons that have a first frequency of use of the reference DNA sequence by codons having a second frequency of use by yeast of greater than or equal to 15 per 1000 codons and encoding the same amino acid as the codon being replaced.

35. The method of claim 34 wherein said first frequency of use is less than or equal to 12 per 1000 codons.

36. The method of claim 34 wherein said first frequency of use is less than or equal to 10 per 1000 codons.

37. The method of claim 34 wherein said codons having said first frequency of use are each independently selected from the group consisting of CTC, CTG and CTT encoding leucine, CGG, CGC, CGA, CGT and AGG encoding arginine, GCG and GCC encoding alanine, GGG, GGC and GGA encoding glycine and CCG and CCC encoding proline.

38. The method of claim 34 wherein said codons having said first frequency of use are each independently selected from the group consisting of CTC and CTG encoding leucine, CGG, CGC, CGA, CGT and AGG encoding arginine, GCG and GCC encoding alanine, GGG and GGC encoding glycine and CCG and CCC encoding proline.

39. The method of claim 34 wherein said second frequency of use is greater than or equal to 18 per 1000 codons.

40. The method of claim 34 wherein said second frequency of use is greater than or equal to 20 per 1000 codons.

41. The method of claim 34 wherein said region of ten consecutive codons comprises at least four codons that have a first frequency of use by yeast of less than or equal 13 per 1000 codons.

42. The method of claim 34 wherein said region of ten consecutive codons comprises at least five codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons.

43. The method of claim 34 wherein said region often consecutive codons comprises at least six codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons.

44. The method of claim 35 wherein at least 50% of said codons that have a first frequency of use are replaced, the replacement selected from CTC, CTG or CTT replaced by a codon independently selected from TTG and TTA; CGG, CGC, CGA, CGT or AGG replaced by AGA; GCG or GCC replaced by a codon independently selected from GCT and GCA; GGG, GGC or GGA replaced by GGT; and CCG or CCC replaced by CCA.

45. The method of claim 35 wherein said region of ten consecutive codons comprises at least three codons that have a first frequency of use by yeast of less than or equal to 13 per 1000 codons.

46. The method of any one of claims 34, 41-43 and 45 wherein at least two of said codons that have a first frequency of use are adjacent.

47. The method of any one of claims 41-43 and 45 wherein at least three of said codons that have a first frequency of use are adjacent.